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Total enzyme activity constraint and homeostatic constraint impact on the optimization potential of a kinetic model

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Abstract

The application of biologically and biochemically relevant constraints during the optimization of kinetic models reduces the impact of suggested changes in processes not included in the scope of the model. This increases the probability that the design suggested by model optimization can be carried out by an organism after implementation of design *in vivo*.

A case study was carried out to determine the impact of total enzyme activity and homeostatic constraints on the objective function values and the following ranking of adjustable parameter combinations. The application of constraints on the model of sugar cane metabolism revealed that a homeostatic constraint caused heavier limitations of the objective function than a total enzyme activity constraint. Both constraints changed the ranking of adjustable parameter combinations: no "universal" constraint-independent top-ranked combinations were found. Therefore, when searching for the best subset of adjustable parameters, a full scan of their combinations is suggested for a small number of adjustable parameters, and evolutionary search strategies are suggested for a large number. Simultaneous application of both constraints is suggested.

Keywords

optimization; kinetic model; adjustable parameters; optimization potential

1. Introduction

The application of mathematical models to achieve biotechnological goals, such as providing necessary fuels, chemicals and materials using metabolic engineering and synthetic biology approaches (Jullesson et al., 2015; Nielsen et al., 2014) is based on engineering principles: new designs are developed based on mechanistic understanding and mathematical description of the process of interest. Several industrial applications have demonstrated the potential of a model-based design approach (Nielsen et al., 2014). At the same time, it is important to determine the smallest number of modifications that have the largest impact on strain improvement (Mozga and Stalidzans, 2014; Nikolaev, 2010; Rodríguez-Acosta et al., 1999; Sendín et al., 2010; Stalidzans et al., 2017b; Stephanopoulos and Simpson, 1997). A small number of manipulated

parameters is advantageous for reducing risks of both unexpected side-effects and costs of model-based design implementation *in vivo*.

The optimization of kinetic models is a popular approach for strain design development. It can be used alone or in combination with larger scale stoichiometric models (Kalnenieks et al., 2014). Kinetic (dynamic) models are usually expressed as a set of ordinary differential equations requesting detailed knowledge about reaction type and parameters and delivering detailed dynamic mechanistic simulations of biochemical networks (Almquist et al., 2014; Stelling, 2004; Villaverde et al., 2016). At the same time, the kinetic models have a drawback, which is their small scale (Almquist et al., 2014), usually up to several tens of reactions. This limits their predictive power. Therefore, applicability of new designs should be validated in larger scale models, for instance, stoichiometric models (Kalnenieks et al., 2014), if possible.

When looking for improvements mostly in the maximum flux and/or yield of product of interest (Sendín et al., 2010; Villaverde et al., 2016) based on a kinetic model, different constraints can be applied to the optimization task settings depending on peculiarities of particular process and/or organism of interest. This can be done in order to reduce the impact of limitations of the organism of interest that are not taken into account by the model due to the small size of model. Ignoring these constraints may lead to overoptimistic expectations, which fail when the proposed changes are implemented by creating modified organisms *in vivo*. In this study we concentrate on two popular constraints that are well discussed and are applicable without transformation of the model.

As first we use constraint mentioned by Waley (Waley, 1964) assuming that the total concentrations of enzymes may be required to remain fixed due to possible limitations of enzyme production resources (amino acids, transcriptional and translational capacity and others). This idea is developed later (Heinrich and Schuster, 1996) and used as total enzyme activity constraint that limits the overexpression of enzymes. Two variants of this constraint are common: i) the total enzyme quantity is fixed at initial or some other value (Magnus et al., 2009; Nikolaev, 2010) or ii) some total quantity can not be exceeded (Klipp et al., 2002; Mauch et al., 2001; Schmid et al., 2004; Stalidzans et al., 2017a).

As second we use the homeostatic constraint (Fell and Thomas, 1995; Kacser and Acerenza, 1993): metabolite concentrations in the steady state of an optimized model may not differ by more than a defined fraction from the steady state metabolite concentrations in the initial model. This constraint limits large changes in metabolite concentrations in the model to avoid their potential impact on other reactions not included in the kinetic model but present in the living organism and to avoid cytotoxicity (Kell and Mendes, 2000). The constraint has variations in its application: i) limitation of metabolite pool total concentration increase (Magnus et al., 2009; Mauch et al., 2001; Nikolaev, 2010; Schmid et al., 2004) without constraining each metabolite individually, ii) limitation of changes of individual metabolites (Rodríguez-Acosta et al., 1999; Stalidzans et al., 2017a; Villaverde et al., 2016) and iii) combination of both (Visser et al., 2004).

In this study, we search for the optimal adjustable parameter sets depending on application of the total enzyme activity constraint and/or the homeostatic

constraint. A high impact of constraints on the best values of objective function and the performance of particular adjustable parameter combinations was observed.

2. Material and methods

2.1. Model, optimization software and task setting

A sucrose accumulation model in sugar cane culm tissue BIOMD0000000023 (Rohwer and Botha, 2001) from the *Biomodels* database was used as a case study (Fig.1) because of well defined optimisation task and handy number of adjustable parameters (AP) for analysis of all possible combinations of AP (31 possible combinations for 5 APs). The *COPASI* software (Hoops et al., 2006) was used as the optimization tool and *SpaceScanner* software (Elsts et al., 2017) was used to manage parallel optimization runs of *COPASI* models. The following global stochastic optimization methods implemented in *COPASI* were used: "Particle Swarm", "Genetic Algorithm", "Genetic Algorithm SR", "Evolutionary programing" and "Evolutionary Strategy SR". *ConvAn* software (Kostromins et al., 2012) was used for visualization and analysis of convergence dynamics of parallel runs. Steady state optimization was used in *COPASI* settings.

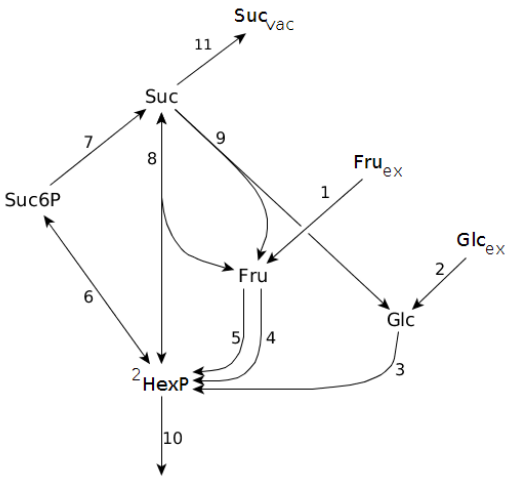

Fig.1 Sucrose accumulation in sugar cane culm tissue (based on Fig.1 (Rohwer and Botha, 2001)). Reactions : 1, fructose (Fru) uptake ; 2, glucose (Glc) uptake ; 3, hexokinase(Glc) ; 4, hexokinase (fructose phosphorylating) ; 5, fructokinase ; 6, sucrose phosphate synthase ; 7, sucrose phosphate phosphatase ; 8, sucrose synthase ; 9, invertase ; 10, glycolysis ; 11, vacuolar sucrose import. Reaction 6 is defined forward in the direction of sucrose 6-phosphate (Suc6P) synthesis, and has a stoichiometry of 2 for HexP (fructose 6-phosphate and UDP-glucose). Reaction 8 is defined forward in the direction of sucrose (Suc) synthesis. The hexose phosphate pool was considered as an equilibrium block comprising UDP-glucose, glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate. HexP, hexose phosphates ; subscript 'ex', extracellular ; subscript 'vac', vacuolar.

Enzyme amount (concentration) factors (Kc) were introduced into the kinetic equations of the model (Bruck et al., 2008). Kc coefficients of reactions 1-5 were used as APs. All Kc values in models before optimization were set to Kc=1. This enabled the normalized implementation of the adjustability range of the enzyme

amount. The range of Kc adjustment during all optimization experiments was between 0.01 and 10, enabling changes within three orders of magnitude:

$$\forall j \in [1; n]: 0.01 \leq Kc_j \leq 10 \qquad (1)$$

where n is the number of adjustable parameters (five in this case) and Kc_j is the enzyme amount factor Kc for reaction j.

The optimization task was set as in the study of model authors Rohwer and Botha (Rohwer and Botha, 2001) and used later also by Mendes and colleagues (Mendes et al., 2009): the objective function (OF) was the maximization of a ratio of fluxes

$$OF = Jv11/jv9 \quad (2)$$

where *Jv* are fluxes of corresponding reactions.

The enzyme concentration coefficients Kc, in reactions *v1, v2, v3, v4* and *v5* are set as AP. The applied OF represents the optimization of the concentration of enzymes to increase the proportion of accumulation in the vacuole (flux *Jv11* in Fig. 1) relative to sucrose hydrolysis by invertase (flux *Jv9* in Fig.1) (Rohwer and Botha, 2001). There were 31 possible combinations of five AP analyzed.

The total optimization potential (TOP) approach (Stalidzans et al., 2017b) is used to assess the biotechnological potential of particular AP combination. TOP is OF value of full set of AP. OF value of any other AP combination can be expressed as a fraction of TOP as all other combinations are just subsets of combination with all AP included.

2.2. Additional constraints

To assess the impact of additional optimization constraints, the *total enzyme activity constraint* was introduced. It was defined as the upper limit of the initial sum of all Kc (initial value of each Kc=1):

$$\sum_{j=1}^{n} \mathrm{K}c_j \leq n \qquad (3)$$

where n is number of adjustable parameters (equals to 5 in this case).

Thus, the initial enzyme production capacity cannot be exceeded, and enzyme concentration can rise only with the cost of corresponding decreases in other enzyme concentration(s) (Klipp et al., 2002; Mauch et al., 2001; Schmid et al., 2004; Stalidzans et al., 2017a).

The homeostatic constraint was implemented by limiting the new steady state metabolite concentrations to a ±20% corridor around the steady state concentrations of each metabolite in the initial model (Rodríguez-Acosta et al., 1999):

$$\forall m \in [1; l]: 0.8c_{om} \leq c_m \leq 1.2c_{om} \quad (4)$$

where l is the number of metabolites, c_m is steady state concentration of a metabolite after optimisation and c_{om} is the are the one of original steady state (original model before optimization).

Steady state solutions where any concentration of any metabolite was outside the defined corridor were rejected by COPASI automatically.

To assess the impact of these additional constraints and their combinations, four different task settings were implemented (see initial *COPASI* models with pre-set optimization tasks for each task setting (TS) in Supplementary material 1):

1) without additional constraints (TS1);
2) total enzyme activity constraint (TS2);
3) homeostatic constraint (TS3);
4) both total enzyme activity and homeostatic constraints (TS4).

3. Results and discussion

Ranked lists of the best solutions according to the OF for TS1–TS4 are summarized in (Table 1). Supplementary material 2 contains the results of optimizations generated by *SpaceScanner* including all Kc values. Columns E-J indicate if Kc of reaction is included in particular combination while columns K-O indicate the Kc values.

Table 1. Ranked list of combinations depending on the task setting.

	Rank				OF value			
Combination	**TS1**	**TS2**	**TS3**	**TS4**	**TS1**	**TS2**	**TS3**	**TS4**
Kc1	28	30	28	30	26.4	3.5	3.81	3.48
Kc2	27	30	27	30	30.8	3.5	3.89	3.48
Kc3	16	16	22	21	8 721.2	8 721.2	3.98	3.98
Kc4	31	28	31	28	3.5	3.5	3.52	3.52
Kc5	30	27	29	26	4.4	4.4	3.77	3.77
Kc1;2	22	29	12	29	312.9	3.5	4.41	3.48
Kc1;3	12	13	16	15	58 506.2	18 740.7	4.10	4.08
Kc1;4	25	25	26	24	35.2	6.5	3.90	3.81
Kc1;5	17	24	23	25	376.1	7.1	3.95	3.80
Kc2;3	10	12	21	20	122 811.0	20 188.8	3.99	3.99
Kc2;4	26	20	25	22	30.8	11.7	3.93	3.93
Kc2;5	24	23	14	10	42.7	8.7	4.38	4.38
Kc3;4	15	15	18	17	13 128.6	13 128.6	4.00	4.00
Kc3;5	14	14	18	17	14 218.5	14 218.5	4.00	4.00
Kc4;5	29	26	29	26	4.5	4.5	3.77	3.77
Kc1;2;3	4	11	6	12	1 381 300.0	24 148.8	4.46	4.20
Kc1;2;4	21	18	11	8	319.5	58.9	4.43	4.41
Kc1;2;5	20	22	4	9	324.3	8.9	4.47	4.39
Kc1;3;4	7	5	13	14	247 027.0	64 552.5	4.40	4.10
Kc1;3;5	11	9	10	13	60 236.5	29 137.3	4.45	4.11
Kc1;4;5	18	21	23	23	372.4	10.6	3.95	3.91
Kc2;3;4	9	8	17	16	131 571.0	39 185.7	4.03	4.03
Kc2;3;5	8	7	5	4	228 685.0	56 426.9	4.46	4.42
Kc2;4;5	23	19	14	10	42.8	23.2	4.38	4.38
Kc3;4;5	13	10	18	17	25 834.0	25 834.0	4.00	4.00
Kc1;2;3;4	2	4	7	2	2 474 050.0	78 996.2	4.46	4.47
Kc1;2;3;5	3	6	3	3	1 430 180.0	61 294.9	4.47	4.46
Kc1;2;4;5	19	17	9	5	331.5	95.5	4.46	4.42
Kc1;3;4;5	5	2	2	7	259 751.0	102 913.0	4.47	4.42
Kc2;3;4;5	6	3	8	6	258 968.0	102 767.0	4.46	4.42
Kc1;2;3;4;5	1	1	1	1	2 600 980.0	160 249.0	4.47	4.47

3.1. Optimization potential

The total optimization potential (TOP) in the analyzed sugar cane model was heavily dependent on the constraints (Table 1). The TOP (best value of OF for the full set of APs) varied in a 10^6 fold range from *2,600,980* (TS1) to *4.47* (TS3 and TS4). Introduction of the homeostatic constraint (TS3) in this model was more influential than adding the total enzyme activity constraint (TS2), as *TOP=4.47* in the case of the homeostatic constraint (TS3), while *TOP=160,249* in the case of TS2. Application of both constraints (TS4) reduced TOP value by less than 0,1% compared to TS3, where only the homeostatic constraint was applied.

The optimization potential per number of adjustable parameters can be analyzed in relative measures as a fraction of TOP (Fig. 2), as well as in absolute values (Fig. 3). Two pairs of task settings behave similarly: those without homeostatic constraint (TS1 and TS2) and those with it (TS3 and TS4). In the case of TS1 and TS2, the optimization potential was utilized gradually when the number of adjustable parameters was increasing (Fig. 2). It occurred more quickly in TS1, while in TS2, the increase of OF depending on the number of involved AP was almost linear (Fig. 2). At the same time, the increase of the OF absolute value for TS1 and TS2 was approximately 10^6-fold (Fig. 3), while in the case of TS3 and TS4, the OF raised from 3.7 (initial model) to 4.47 (increase of OF by 22%), but the TOP was almost reached by just two adjustable parameters in TS3 and TS4.

The high TOP values of TS1 and TS2 differed from the relatively small numbers for TS3 and TS4, where the homeostatic constraint was implemented, possibly because of unfeasibly high levels of fructose and glucose concentrations in the optimal solutions of TS1 and TS2 (Table 2), which are not accepted when the

homeostatic constraint is implemented. The heavy impact of the homeostatic constraint could be reduced by setting different constraint values for particular metabolites after assessment of their concentration influence on the rest of biochemical network. For instance, concentration changes of metabolites that are involved in just one metabolic pathway may be constrained less than highly interconnected metabolites.

The impact of homeostatic constraint corridor width (initially ±20%) of all internal metabolites on TOP values in TS3 (Fig. 4) demonstrates linear dependence. Corridor 0% does not allow any increase of TOP and there is a linear growth of OF up to 5-fold OF increase (up to 17.5) at 200% corridor. The curve is similar to the OF dependence on deviation from pool concentration in the study of Mauch and colleagues (Mauch et al., 2001).

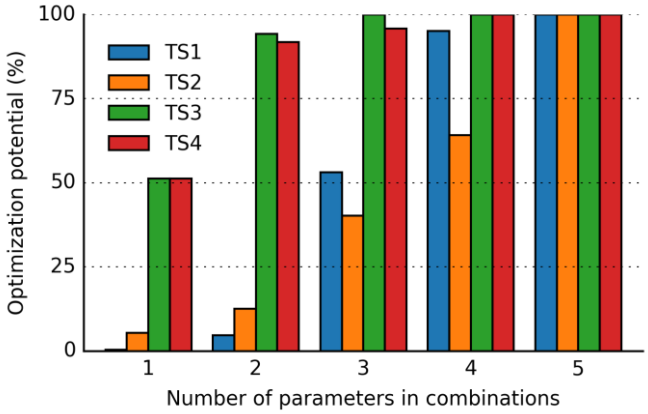

Fig. 2. Optimization potential per number of adjustable parameters.
In all TS, 0% of OF is 3.7 (OF value of initial model) while 100% of OF (TOP) corresponds to OF values of 2,600,980; 160,249; 4.47 and 4.47 for TS1; TS2; TS3 and TS4, respectively.

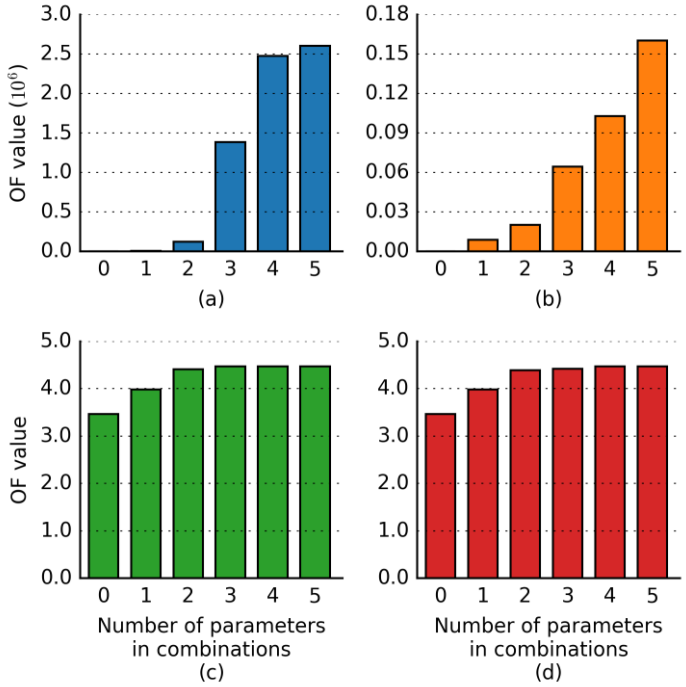

Fig. 3. Optimization potential per number of adjustable parameters in absolute numbers for TS1 (a), TS2 (b), TS3 (c) and TS4 (d).

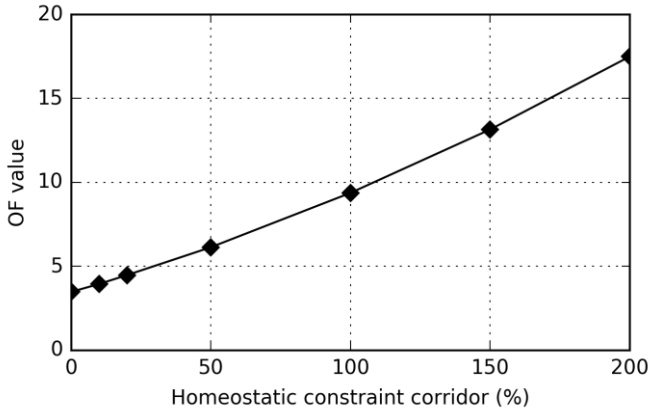

Fig.4. TOP dependence on the homeostatic constraint corridor.

Table 2. Metabolite steady state concentrations (mmol/l) and their changes compared to the initial model in % for the full set of (5 AP) optimizations for TS1–TS4.

Metabolite	Initial	TS1	%	TS2	%	TS3	%	TS4	%
Fru	40.58	47 705	117 455	11 820	29 027	48.70	20	48.70	20
Glc	30.11	45 674	151 588	11 335	37 545	36.13	20	36.13	20
HexP	2.99	0.0031	-100	0.0032	-100	3.43	15	3.44	15
Suc6P *(×10^{-3})*	4.78	1.55E-5	-100	1.58E-5	-100	5.72	20	5.73	20
Suc	10.41	0.1488	-99	0.1489	-99	12.25	18	12.25	18
Sum of Kc		*20.03*		*5.00*		*6.60*		*4.89*	

3.2. Impact of particular reactions to the objective function

In the cases of TS1 and TS2 in all AP combinations, the coefficients Kc5, Kc3 and Kc4 had to be reduced, while Kc1 and Kc2 had to be increased to improve the OF values. Another feature of the TS1 and TS2 ranked solutions list is the high impact of Kc3: all cases with reduced Kc3 had top ranks with 10–100 fold higher OF values compared to the other solutions. These kinds of improvements might be suggested intuitively without optimization activities. The same suggestions were made by model authors (Rohwer and Botha, 2001) using metabolic control analysis (MCA) approach (Heinrich and Rapoport, 1974; Kacser and Burns, 1973).

However, the situation was different in TS3 and TS4, where the homeostatic constraint was implemented, and steady state solutions with metabolite values with more than 20% difference from the initial model were rejected. In this case, coefficients Kc1–Kc5 had much smaller deviations from their initial values and relative changes of Kc3 did not exceed 27%. Simplistic rules like "Kc3 always has to be reduced" were not applicable even in the relatively small model used in this study. Thus, implementation of homeostatic constraint change the solution space the way that MCA predictions that are valid for TS1 and TS2, can not help to predict the most influential changes. Evidence for that is the fact that only Kc1 in TS3 is upregulated and Kc4 in TS4 is downregulated in all combinations (see Supplementary material 2). All other Kc values in TS3 and TS4 are upregulated and downregulated depending on the particular combination of AP. This complex behavior may evolve when changes of enzyme concentration help to neutralize the impact of other enzymes on the concentration changes of an internal metabolite. Similar limitations of MCA after implementation of homeostatic constraint have been reported also before (Kell and Mendes, 2000; Visser et al., 2004).

3.3. Dependence of Kc values on constraints

Regarding the sum of Kc coefficients (sum of Kc1–Kc5 equals 5 at the beginning), it is interesting to observe that the implementation of the homeostatic constraint (TS3) led to a heavy reduction in the sum of coefficients in many AP combinations (Fig.5b): average sum of Kc from 12.7 in TS1 dropped to 6.8 in TS3 just because of limitations of metabolite changes. At the same time, there were solutions in TS3 with high sums of Kc (up to 14.1) indicating necessity to apply homeostatic constraint in combination with the total enzyme activity constraint to limit the sum of Kc.

3.4. Ranking of adjustable parameter combinations depending on constraints

If the rank of AP combinations was independent of the type of constraints, there would be an opportunity to determine the best combinations without additionally constraining the optimization task. Our results show that ranking according to OF values of particular combinations changed quite radically depending on applied constraints (Fig.5a). For example, the combination of Kc1 and Kc2 in task TS3 reached 94% of TOP, while in other task settings it was below 1% of the TOP.

As reported in section 3.2, Kc3 had a high impact on the objective function in TS1 and TS2 being in combination with other Kc, while alone it had a very low contribution to the optimization potential: less than 1% for TS1 and 5% for TS2 (Fig.5a). Conversely, for tasks TS3 and TS4 the Kc3 alone reached 51% of the TOP (Fig.5a).

Another notable case is the combination of Kc1, Kc2, Kc4 and Kc5. For TS1 and TS2, this combination had low optimization potential (0.01% and 0.06%, respectively), while in TS3 and TS4, it resulted correspondingly in 98% and 96% of the optimization potential.

In the case of our test model, we did not find “universal” AP combinations that were highly ranked independent on constraints. Thus, examining all combinations for all TS was appropriate for finding the best AP combinations. A full search could be replaced by an evolutionary algorithm-guided search in the case of a combinatorial explosion of the AP combinations.

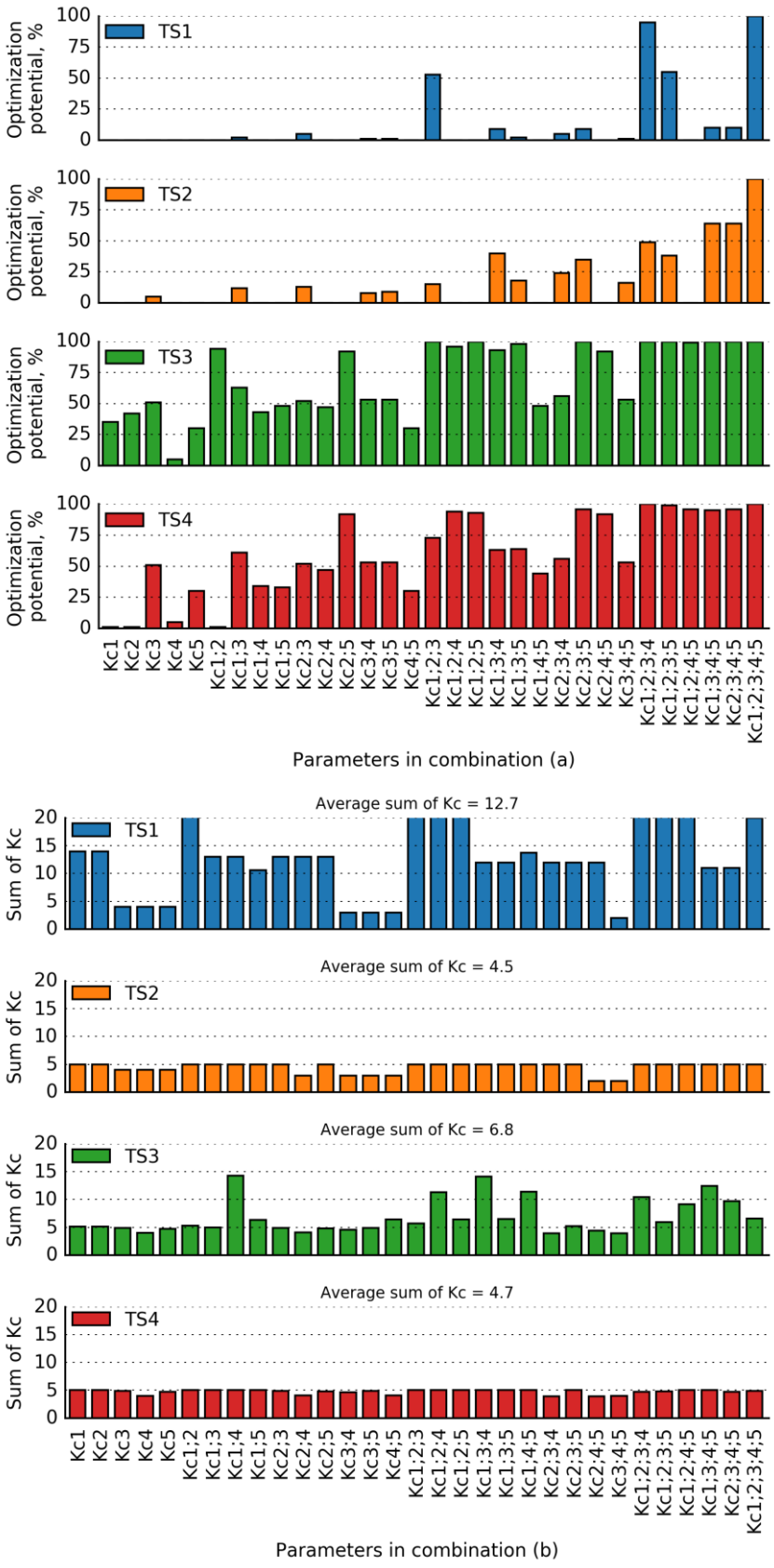

Fig. 5. Fractions of TOP (a) and sum of K1..Kc5 (b) for optimization results of particular AP combinations.

4. Conclusions

Adding constraints to the minimal setting of the optimization task improved the feasibility probability of the solutions found, by heavily reducing the solution space and consequently the objective function value. Implementing constraints is a valuable route to the early assessment of small kinetic model-based design incompatibilities with the complexity of processes in the organism. As a result high-risk designs can be rejected at the modeling level before they have been implemented and found to fail *in vivo*. Still, feasibility of design can be tested just in biological experiments. Even heaviest constraints can not guarantee success of implementation.

The high impact of the homeostatic constraint suggests that careful analysis of acceptable concentrations of particular metabolites needs to be performed. By doing so, opportunities to soften the impact of the homeostatic constraint, through more accurate implementation, may be found. A similar implementation of protein size and initial concentration related relative costs of overexpression would also lead to more accurate implementation of the enzyme activity constraint.

Homeostatic constraint indirectly reduces also the total enzyme activity. Still, some optimization results without total enzyme activity constraint request increase of enzyme concentration very likely above physiological limitations of organism. Therefore application of both constraints is suggested.

We did not find “universal” adjustable parameter combinations that would be highly ranked with examined types of constraints. A full scan of adjustable parameter combinations is suggested, with the aim of finding a small and efficient set of adjustable parameters. Alternatively, the total optimization potential approach (Stalidzans et al., 2017b) or evolutionary search strategies may be applied.

The optimization results presented here should not be used as metabolic engineering designs for the improvement of sugar accumulation in sugar canes without preliminary analysis of their stability or practical implementability from a physiological point of view.

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